BIO-FLOTATION HARVESTING OF *CHLORELLA VULGARIS* MICROALGAE USING *JATROPHA CURCAS* L. PROTEIN-OIL EMULSION

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SUPERVISOR'S DECLARATION

I hereby declare that I have checked this thesis, and, in my opinion, this thesis is adequate in terms of scope and quality for the award of degree of Bachelor of Engineering Technology in Energy & Environmental.

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries in which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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ABSTRACT

This study is a whole new approach to recover microalgae from aqueous medium using bio-flotation with its effectiveness examined. This new bio-flotation method involves utilizing a *Jatropha curcas* protein extract-oil emulsion mixed with sunflower oil for flotation removal of *Chlorella vulgaris* microalgae from its culture medium. Asia, particularly Japan and Taiwan are the largest producer country for *Chlorella vulgaris* in the world and different uses of this microalgae is being discover from time to time involving food, medical, aquaculturally, water treatment, skincare, and biofuel industry. Whereby, the dewatering stage of microalgae production is always a critical issue. Researches and comparison have been done among current common dewatering method including coagulation, flocculation, centrifugation, sedimentation, and many more to improvise this study. This project covered the process from microalgae cultivation, protein extraction of *Jatropha* seed, oil emulsion testing to flotation experiment under various parameters. The effectiveness of this method under various factors has been determined, including operating parameters such as pH, protein-oil emulsion dosage, and mixing time. A maximum flotation efficiency of 81% was achieved under protein-oil emulsion dosage of 20ml/L, pH 2, mixing time 4 min. Furthermore, the change of zeta potential of the microalgae is analysed and showed a mark difference in value affected by the protein-oil emulsion. This flotation method is not only simple, low cost, environmentally friendly, but also an efficient method for harvesting microalgae from culture medium. For the future scope, improvement should be focus on the pH limitations to enhance the feasibility of this method for large scale industrial implementation.

ABSTRAK

Kajian ini merupakan pendekatan baru untuk mendapatkan mikroalga dari medium berair menggunakan bio-flotation dengan keberkesanannya diperiksa. Kaedah bio-flotation yang baru ini melibatkan penggunaan emulsi minyak ekstrak buah *Jatropha curcas* bercampur dengan minyak bunga matahari untuk penyingkiran pengapungan mikroalgae *Chlorella vulgaris* dari medium berairnya. Asia, terutamanya Jepun dan Taiwan merupakan negara pengeluar terbesar untuk *Chlorella vulgaris* di seluruh dunia dan pelbagai kegunaan mikroalga ini sedang ditemui dari semasa ke semasa yang melibatkan bidang makanan, perubatan, akuakultur, rawatan air, penjagaan kulit dan industri biofuel. Dengan ini, peringkat penyahairan pengeluaran mikroalga sentiasa menjadi isu kritikal. Penyelidikan dan perbandingan telah dilakukan di kalangan kaedah penyahairan umum seperti koagulasi, pemberbukuan, sentrifugasi, pemendapan, dan banyak lagi untuk menambah baik kajian ini. Projek ini meliputi proses dari penanaman mikroalgae, pengekstrakan protein benih *Jatropha*, ujian emulsi minyak kepada percubaan pengapungan di bawah pelbagai parameter. Keberkesanan kaedah ini di bawah pelbagai faktor telah dikajikan, termasuk parameter operasi seperti pH, dos emulsi minyak-protein, dan masa pencampuran. Kecekapan pengapungan maksima sebanyak 81% dicapai di bawah dos emulsi minyak-protein 20ml / L, pH 2, masa pencampuran 4 min. Tambahan pula, perubahan potensi zeta mikroalgae dianalisis dan menunjukkan perbezaan nilai di bawah pengaruh emulsi minyak-protein. Kaedah pengapungan ini bukan sahaja mudah, kos rendah, mesra alam, tetapi juga kaedah yang efisien untuk menuai mikroalga dari medium berairnya. Untuk skop masa depan, penambahbaikan harus menumpukan kepada batasan pH untuk meningkatkan kemungkinan kaedah ini untuk pelaksanaan industri skala besar.

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LIST OF SYMBOLS

cm	Centimetre
°C	Degree Celsius
kg	kilogram
L	litre
m	Meter
µm	Micro meter
ml	Millilitre
mm	Millimetre
min	Minute
%	percent
mg	milligram
d	day
g	gram
h	hour
m^3	volume in meter
m^2	area in meter
v	volume
ppm	parts per million
Mpa	megapascal
rpm	revolutions per minute
nm	nanometre
µ	specific growth rate

LIST OF ABBREVIATION

USA	United State of America
PUFAs	poly-unsaturated fatty acids
SPT	Spiral plate technology
CO_2	Carbon dioxide
Fe_{3+}	Iron cation
Al_{3+}	Aluminium cation
JPOE	*Jatropha* protein-oil emulsion
C.vulgaris	*Chlorella vulgaris*
NA	Not available
BBM	Bold's Basal Medium
O_2	Oxygen
J.curcas	*Jatropha curcas*
H_2O	water
UMP	Universiti Malaysia Pahang
DI water	Deionized water
H_2SO_4	Sulphuric acid
KOH	Potassium hydroxide
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1. PROJECT BACKGROUND

Increasing in population is good news for human community, the current average population increase is estimated at 83 million people per year (Worldometers, 2018). However, what come behind is the high demand of resources. Fear that we are running out of important resources is perpetual. Oil is a favourite thing to worry about; landfill space is another, and trees yet another (Lee, 2000).

Therefore, microalgae are gradually become more popular in choice of cultivation in multiple industry field, which provide much more benefits more than a regular cultivation crop. Microalgae is a type of single-celled organisms which able to store significant amounts of energy rich compounds used in biofuels production, such as biodiesel and ethanol. Secondly, they can grow up to twice as fast as conventional biofuels crops. Thirdly, they do not require as much resources, aside from a few nutrients, and water. And lastly, they are able to grow in non-arable lands such as waste-water, sea-water, and certain nutrient-deficient environments (HuLAB, 2012).

In the context of a sustainable bio-economy, microalgae are promising candidates to compensate for greenhouse gas emissions due to their high capability for fixing carbon dioxide. Microalgae are generally eukaryotic organisms, although cyanobacteria, such as spirulina, which are prokaryotes, are included under microalgae due to their photosynthetic and reproductive properties. Microalgae range in size from about 5 μm (*Chlorella*) to more than 100 μm (*spirulina*).

The commercial cultivation of microalgae began in Japan with the cultivation of *Chlorella* in the 1960s, followed by the cultivation of spirulina in Mexico and the USA in the 1970s. Since then, the industrial biotechnology of microalgae has grown tremendously. The immense chemical diversity of microalgae provides numerous

applications in the food, feed and pharmaceutical industries. Microalgae are cultivated for the production of whole biomass and valuable substances such as nutraceuticals, carotenoids, phycocyanin and poly-unsaturated fatty acids (PUFAs), which are utilized in the food and feed (notably aquaculture) industry. The production of biofuel from lipid- or carbohydrates-rich microalgae is under way (Feedipedia - Animal Feed Resources Information System, 2017).

Commercial production of microalgae already has at least 30 years of history. Main species of microalgae which being grown are *Chlorella, Spirulina, Dunaliella salina* and *Haematococcus pluvialis*. Initially these species of microalgae were grown commercially for purpose of food supplement and pharmaceutical industry and currently developing towards renewable fuel source (Sathe).

Microalgae cultivation is the stage of growing microalgae which will contribute up to 30% of total production cost. In commons, there is two major system of microalgae which is open system and closed system. Open system, also named as open-pond system a more common and low-cost method which normally used in mass cultivation. However, it has a major drawback which is easily contaminated by bacteria and unpredictable environmental factors including evaporation, light intensity, pH levels and temperature which bring negative effect of biomass concentration. Therefore, the second cultivation method is by using closed system. Closed system grows microalgae inside a photobioreactor. This allow the control of environmental factors for a more efficient growth. Despite that, this method will have required cost in bioreactor construction and energy demands for operation (HuLAB, 2012).

For the harvesting, microalgae are needs to be concentrated and separates from the growth medium. Harvesting can be divided into two-step process which first is bulk harvesting and followed by thickening process. In the bulk harvesting, microalgal biomass is separated from the bulk cultures. While in the thickening process, it is to concentrates the algal slurry. Generally, method of harvesting microalgae is divided into 4 based methods involving mechanical, chemical, biological and electrical methods.

Mechanical technique is including centrifugation, filtration, gravity sedimentation, flotation, and foam separation. For chemical dewatering, this method is mostly flocculation induced by either inorganic or organic polyelectrolyte (polymer)

flocculants. While for electrical dewatering, this method is based on electro-coagulation process. For biological dewatering technique, it includes auto-flocculation which occurring at high pH, flocculation by secreted biopolymers, and microbial flocculation.

Nowadays, extensive research has been done on different microalgae species in regards of the economic and technical limitation on microalgae harvesting. Separation of microalgae from their aqueous medium is a critical steps which accounts up to 30% of total biomass production cost (Salim, Bosma, Vermuë, & Wijffels, 2011). Current harvesting methods is energy cost extensive, toxicity from the flocculants and not feasible in the large-scale production. However, in the current technology, different type of technique has shown a remarkable potential to be carry out for harvesting and dewatering microalgae.

This technology is complicated due to the both physical and chemical properties of dilute algal solution. By using a high impact performance single technology, or combination technique in sequence, harvesting and dewatering microalgae can be carrying out. The effectiveness from the combination technique in sequences is relying on the individual performances for each unit. The first combine technique performances will affect the following technique in the combination. Example of the current available technology for harvesting and dewatering for microalgae is centrifugation, spiral plate technology (SPT), pressure filtration, vacuum filtration, membrane filtration, sedimentation, chemical flocculation, drum drying, spray drying and solar drying.

For solid-liquid separation, it can using different method such as sedimentation, disc stack centrifugation, dissolved air flotation, dispersed air flotation, micro bubble generation organic flocculation, inorganic flocculation, vacuum filtration, cross flow filtration, pressure filtration, decanter centrifugation, bio-flocculation, auto-flocculation and electrolytic coagulation, electrolytic flocculation and electrolytic flotation (Al Hattab, Ghaly, & Hammoud, 2015)

Jatropha curcas is a species of flowering plant in the spurge family, *Euphorbiaceae*, native to the American tropics most likely Mexico and Central America and has been spread throughout the world in tropic and subtropical regions around the world and make the cultivation of *Jatropha* uncomplicated (Ab van Peer, n.d.) .The plant can grow in wasteland and grow on almost any terrain even on gravelly, sandy and saline

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